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14. ABSTRACT

A large percentage of prostate cancers show either loss or mutational inactivation of the Rb tumor suppressor gene. Rb mediates its tumor suppressor function through its association with other cellular proteins. Our study focuses on Thoc1 protein, which interacts with the N terminal region of Rb protein and thereby may mediate some Rb functions. Previous reports show that Thoc1 is upregulated in some cancer types and is required for survival of transformed cells, suggesting Thoc1 may play a role in tumorigenesis. To test our hypothesis, we are using a mouse model of prostate cancer where prostate-specific deletion of Rb and p53 genes leads to development of metastatic adenocarcinoma. We find that deletion of Thoc1, Rb and p53 genes leads to increased survival of mice and delayed development of prostate tumor lesions compared to mice with loss of Rb and p53. Deletion of Thoc1 in cells isolated from the prostate tumors resulted in significant decrease in clonogenicity and loss of viability compared to cells retaining Thoc1 expression. Interestingly, decreased amounts of Thoc1 protein alone in the prostate gland of the mouse did not affect normal prostate gland development. The differential requirement for Thoc1 in tumor cells compared to normal cells suggests that Thoc1 may be a potential target for prostate cancer therapy..

15. SUBJECT TERMS
Thoc1, Rb1, transcription elongation, mouse models

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Introduction

Despite major breakthroughs in the early diagnosis and therapy over the last 50 years, prostate cancer remains the second leading cause of cancer death in men in the U.S. New approaches in prevention, diagnosis and therapeutic intervention are required. Prostate tumors harbor mutations or loss of *Rb1* tumor suppressor gene in nearly half of the cases (Phillips et al., 1994). The *Rb1* gene product, pRb, mediates its cellular function through its association with other cellular proteins. Most of the pRb-binding proteins such as E2F family of transcription factors, viral oncoproteins such as E1A, interact with pRb through the pocket domain located on its C-terminal region. More than 150 binding partners of pRb have been reported in the literature and only a handful of those proteins are known to interact with the amino terminal region of pRb (Goodrich, 2003). The amino terminal region of pRb has been shown to be essential for the tumor suppression function of pRb *in vivo* and also for mouse embryonic development (Xu et al., 1994; Riley et al., 1997). These observations suggest that some of the cellular functions of pRb may be mediated through its N terminal binding partners such as Thoc1, particularly in the context of tumor suppression and mouse embryonic development.

Thoc1 protein is an essential component of THO sub-complex of the larger TREX (transcription/export) complex which physically couples transcription elongation with RNA processing and export from the nucleus, thereby involved in the regulation of a sub-set of genes (Li et al., 2005, Guo et al., 2005). Thoc1 protein has been found to be upregulated in prostate cancers (unpublished data), in prostate tumor cells derived from the TRAMP (transgenic adenocarcinoma of mouse prostate) mouse model of prostate cancer (unpublished data) and in other cancer types such as breast cancer (Guo et al., 2005) and non-small cell lung cancer (Yang et al., 2008) suggesting that Thoc1 may play a role in tumorigenesis. We also found that oncogene transformed mouse embryonic fibroblasts (MEFs) were dependent on Thoc1 for their survival whereas normal MEFs did not require Thoc1 for survival (Li et al., 2007).

Although Thoc1 protein physically interacts with pRb, the functional significance of their interaction in the context of tumorigenesis is not known. *Rb1* and *Thoc1* seem to have opposite effects on cellular physiology in tumor cells. For example, *Rb1* is either mutated or lost in prostate cancers whereas *Thoc1* has been found to be upregulated in prostate tumors suggesting that they may interact in an antagonistic manner. Based on this, we propose to determine if *Rb1* and *Thoc1* functionally interact in prostate tumorigenesis and if Thoc1 plays a role in the initiation and development of adenocarcinoma and metastasis using a mouse model of prostate cancer.

Body

Research and Training Accomplishments

Task 1. Test if Rb and Thoc1 functionally interact in mouse prostate tumorigenesis.

All the studies pertaining to Task 1 were completed and reported in the previous years.

Task 2. Determine whether Thoc1 mediates mouse prostate adenocarcinoma and metastasis.

To determine if Thoc1 plays a role in mouse prostate tumorigenesis, we used a mouse model where deletion of *Rb1* and *p53* genes in the prostate epithelium leads to metastatic adenocarcinoma of the prostate (Zhou et al, 2006). We crossed $Rb1^{F/F}$:$p53^{F/F}$:*PB-Cre4* mice with $Thoc1^{F/F}$ mice to generate $Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{F/F}$:*PB-Cre4* (test genotype) and $Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{+/+}$:*PB-Cre4* (control genotype) mice. We found that $Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{F/F}$:*PB-Cre4* mice (test genotype) had significantly longer survival and developed prostate tumors with increased latency compared to the mice in control genotype group suggesting that *Thoc1* is required for tumorigenesis process initiated by loss of *Rb1* and *p53* in the mouse prostate. All the prostate tumor lesions in the mice of the test genotype retained the expression of Thoc1 protein due to inefficient recombination of *Thoc1* floxed allele.

We next wanted to study the mechanism underlying the inhibition of prostate tumor development upon loss of *Thoc1*. Prostate tumor cells were isolated from primary prostate tumors and cultured *in vitro*. The cells of the test genotype retained at least one unrecombined *Thoc1* allele. Both alleles of *Rb1* and *p53* were recombined in the test and control genotypes. To study the effect of *Thoc1* loss on clonogenicity, cells were infected with Ad5-CMV-Cre-eGFP to achieve complete deletion of *Thoc1*. Ad5-CMV-eGFP served as a control for the effects of adenoviral infection. Cells of the positive control genotype ($Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{+/+}$:*PB-Cre4*) were similarly treated. Complete recombination of the *Thoc1* floxed allele and a decrease in *Thoc1* mRNA was observed after Ad5-CMV-Cre-eGFP infection of $Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{F/F}$:*PB-Cre4* cells (Fig. 1A and B respectively). Ten days after initial adenoviral

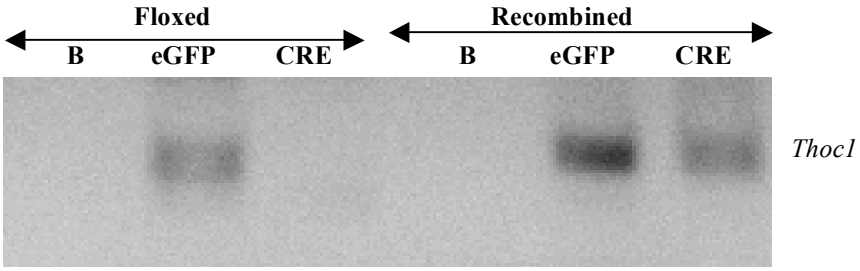
infection, loss of *Thoc1* significantly decreased the clonogenic ability of prostate tumor cells suggesting that *Thoc1* is required for clonogenic propagation of tumor cells (Fig. 2A and B).

We next tested if *Thoc1* deletion caused apoptotic cell death in prostate tumor cells. Prostate tumors cells of the test and positive control genotypes were infected with Ad5-CMV-Cre-eGFP and Ad5-CMV-eGFP. Two days after viral infection, cells were incubated with APC-Annexin V, which has a high affinity for phosphotidylserine that is translocated to the outer cell membrane in cells undergoing apoptosis. Cells were analyzed by Flow cytometry to identify cell populations that were GFP and Annexin V positive. Deletion of *Thoc1* significantly increased apoptosis of prostate tumor cells of the test genotype compared to the positive control cells (Fig. 3). The above findings indicate that *Thoc1* is required for clonogenicity and survival of prostate tumor cells.

We also studied the effect of decreased levels of Thoc1 protein alone on the mouse prostate development and found that loss of Thoc1 in the prostate did not affect the normal prostate gland development. These findings suggest that while *Thoc1* is required for mouse prostate tumorigenesis initiated by prostate-specific deletion of *Rb1* and *p53* genes, Thoc1 is dispensable for normal development of the mouse prostate gland.

Working on the above tasks gave me an opportunity to learn various research techniques such as PCR genotyping, real time PCR, FACS analysis, H&E staining, immunohistochemistry, histopathological analysis of lesions in prostate, liver and lungs tissues and mouse husbandry. In addition, I got an opportunity to work on the design of the experiments, analysis and interpretation of data, skills that are highly important for my training. My training program also included attending weekly seminar meetings of the Genitourinary Program at Roswell Park Cancer Institute. Investigators from within Roswell Park Cancer Institute and invited speakers from other institutes working on different topics of prostate cancer research present their work at these seminars, which has helped broaden my understanding of the disease as well as of the research in the field of prostate cancer. I also got an opportunity to present a poster based on the above findings at the DOD sponsored Innovative Minds in Prostate Cancer Today (IMPaCT) conference 2011 in Orlando, FL. Attending this meeting gave me an opportunity to present and discuss my research with researchers from other institutions as well as consumer advocates. With the help of this training grant I completed the requirements for obtaining a Ph.D degree in Molecular Pharmacology and Cancer Therapeutics from Roswell Park Graduate Division of the University at Buffalo, The State University of New York, Buffalo, NY in September 2010.

A.



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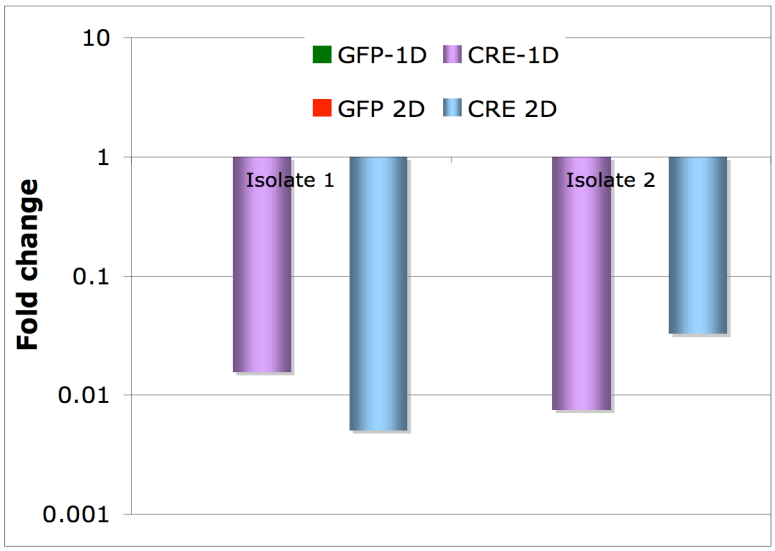
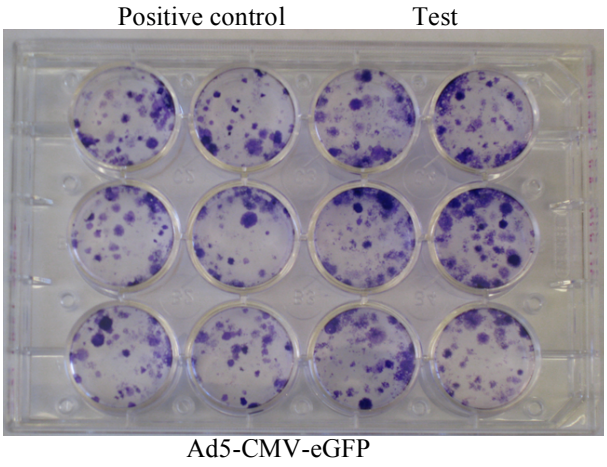
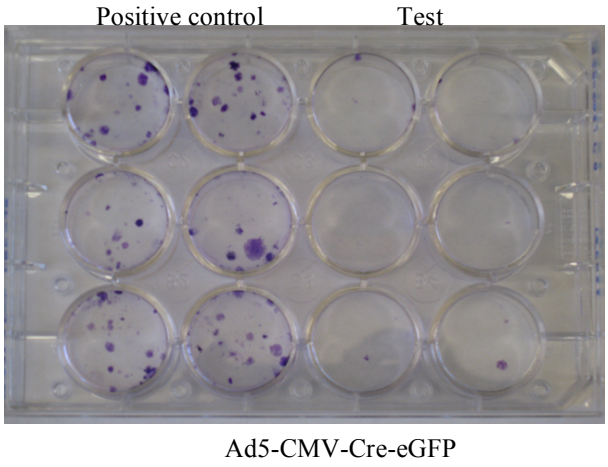


Figure 1. Analysis of prostate tumor cells of the *Rb1*$^{F/F}$*:p53*$^{F/F}$*:Thoc1*$^{F/F}$*:PB-Cre4* genotype post adenoviral infection. A) PCR genotyping shows complete recombination of *Thoc1* allele upon adenoviral cre infection. B) Real time RT-PCR analysis of *Thoc1* mRNA shows decreased *Thoc1* expression upon adenoviral cre expression. 1D=1 day post adenoviral infection; 2D=2 days post adenoviral infection. eGFP/GFP=Ad5-CMV-eGFP infection; CRE= Ad5-CMV-Cre-eGFP infection. The fold change in *Thoc1* expression for GFP 1D and GFP 2D group has been set as 1. The fold change in *Thoc1* expression in CRE 1D and CRE 2D is relative to corresponding GFP group. Results from two independent isolates (Isolate 1 and 2) of cells are shown here.

A.





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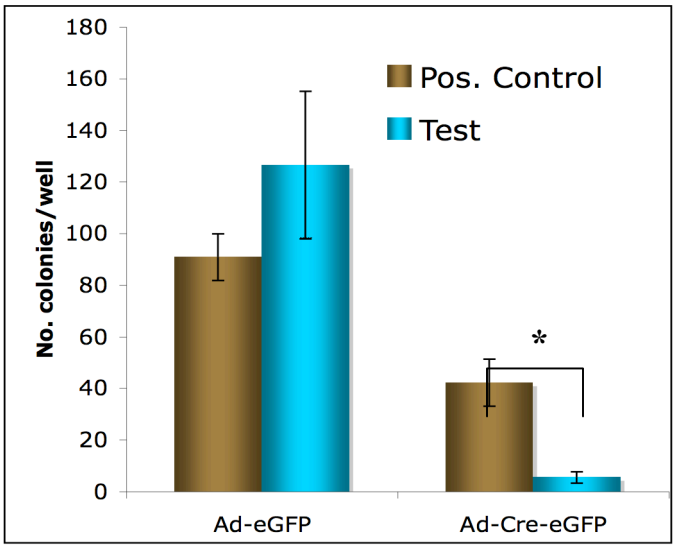

Figure 2. Effect of *Thoc1* deletion on clonogenicity of prostate tumor cells. A) Crystal violet-stained colonies after 10 days of plating Ad5-CMV-Cre-eGFP or Ad5-CMV-eGFP infected cells (1000cells/well) show decreased clonogenicity upon loss of *Thoc1* (test genotype). B) Quantitation of colonies in plate A revealed a significant decrease in clonogenicity upon loss of *Thoc1*. A modest decrease in clonogenicity in cells having wild type *Thoc1* was perhaps due to non-specific toxicity of adenovirus cre. n=6/genotype. * denotes, Student's *t*-test, $p<0.001$. Positive control = $Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{+/+}$:*PB-Cre4*; Test = $Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{F/F}$:*PB-Cre4*.

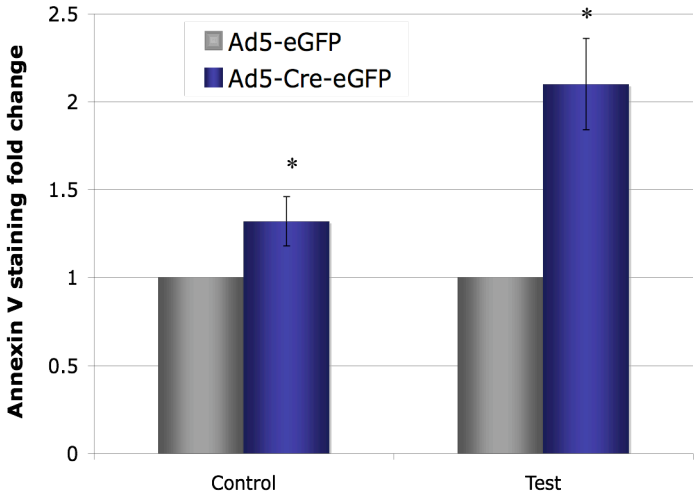

Figure 3. Effect of *Thoc1* loss on viability of prostate tumor cells. Loss of *Thoc1* upon Ad5-CMV-Cre-eGFP infection caused a significant increase in apoptosis (test group) compared to cells retaining *Thoc1* (positive control). n=4/genotype. * denotes statistically significant difference in apoptotic cells between Ad5-CMV-Cre-eGFP infected test ($Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{F/F}$:*PB-Cre4*) and positive control ($Rb1^{F/F}$:$p53^{F/F}$:$Thoc1^{+/+}$:*PB-Cre4*) group, Student's *t*-test, $p<0.05$.

Key Research Accomplishments

1. *Thoc1* is required for mouse prostate tumorigenesis initiated by prostate specific loss of *Rb1* and *p53*.
2. *Thoc1* is required for clonogenicity and survival of mouse prostate tumor cells.
3. Loss of *Thoc1* alone in the mouse prostate did not affect the normal development of the prostate gland.

Reportable Outcomes

1. Meenalakshmi Chinnam earned a Ph.D degree in Molecular Pharmacology and Cancer Therapeutics from Roswell Park Graduate Division of the University at Buffalo, The State University of New York, Buffalo, NY, September 2010.

2. Meenalakshmi Chinnam, Yanqing Wang, Xiaojing Zhang, David L. Gold, Thaer Khoury, Alexander Yu Nikitin, and David W. Goodrich. Thoc1 is required for mouse prostate tumorigenesis initiated by prostate specific loss of Rb and p53. Innovative Minds in Prostate Cancer Today (IMPaCT) conference, March 2011, Orlando, FL.

Conclusion

Thoc1 protein is a pRb–associating protein that has been found to be upregulated in breast, lung and prostate tumors. Therefore, Thoc1 may play a role in tumorigenesis. Studies from our lab have shown the requirement of Thoc1 protein in the survival of E1a/Ras transformed cells and cells of numerous human cancer cell lines. These findings suggest that transformed cells may require Thoc1 for their survival. To study the role of Thoc1 in tumorigenesis process, we used a mouse model of prostate cancer and found that loss of *Thoc1* specifically in the prostate epithelium significantly increased the survival of mice and delayed the development of prostate tumors. Any tumors that developed in the Thoc1 deleted group retained the expression of Thoc1 protein due to incomplete knock out of the Thoc1 gene. Inhibition of tumor formation in the absence of a functional *Thoc1* gene may have been due to the loss of viability of cells upon deletion of *Thoc1* gene. Loss of *Thoc1* by itself did not affect the normal development of the prostate gland in the mouse. The differential requirement for *Thoc1* in mouse prostate tumorigenesis but not for normal prostate gland development makes Thoc1 a potential target for prostate cancer therapy.

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